

**PATENT APPLICATION**  
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of

Docket No: Q83408

Kousuke TANI, *et al.*

Appln. No.: 10/506,536

Group Art Unit: 1626

Confirmation No.: 1208

Examiner: Chung, Susannah Lee

Filed: September 03, 2004

For: 8-AZAPROSTAGLANDIN DERIVATIVE COMPOUNDS AND DRUGS  
CONTAINING THE COMPOUNDS AS ACTIVE INGREDIENT

**DECLARATION UNDER 37 C.F.R. § 1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Tsutomu Shiroya, hereby declare and state:

THAT I am a citizen of Japan;

THAT I have received the degree of a Master of Science in 1989 from Kyoto  
Pharmaceutical University;

THAT I have been employed by Ono Pharmaceutical Co., Ltd. since 1989, where I  
hold a position as HEAD of pharmacology Group 3, with responsibility for study of  
prostaglandin at the Research Institute of Ono Pharmaceutical Co., Ltd.;

THAT this declaration is made for support of the above identified U.S. Patent  
Application;

THAT I am a co-inventor of the present application; and

THAT the following experiments (Activity of EP<sub>2</sub> Receptor Agonist) were conducted  
under my direct supervision.

**EP<sub>2</sub> Receptor Binding Activity**

Experiment for measurement of the an EP<sub>2</sub> receptor binding activity with the  
cells expressing prostanoid receptor sub-types

According to the method of Sugimoto *et al.* (*J. Biol. Chem.*, 267, 6463-6466 (1992)), CHO cells which expressed prostanoid receptor sub-types (murine EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3α</sub>, and EP<sub>4</sub>, respectively) were prepared and used as membrane authentic samples.

A reaction solution (200 μl) containing the prepared membrane fraction (0.5 mg/ml) and <sup>3</sup>H-PGE<sub>2</sub> was incubated at room temperature for 1 hour. The reaction was terminated with ice cold buffer (3 ml), and the reaction mixture was filtered under suction through a glass filter (GF/B), on which the binding <sup>3</sup>H-PGE<sub>2</sub> was trapped. The binding radioactivity was measured by means of a liquid scintillator.

The K<sub>d</sub> value was obtained from the Scatchard plots [*Ann. N.Y. Acad. Sci.*, 51, 660 (1949)]. Non-specific binding was obtained as the binding in the presence of an excess amount (2.5 μM) of unlabelled PGE<sub>2</sub>. Measurement of the binding inhibition for <sup>3</sup>H-PGE<sub>2</sub> with the compounds of the present invention was performed by adding <sup>3</sup>H-PGE<sub>2</sub> (2.5 nM) and a series of concentrations of the compound of the present invention. In this reaction, the following buffer was used in all cases.

Buffer: 10 mM potassium phosphate (pH 6.0), 1 mM EDTA, 10 mM MgCl<sub>2</sub>, and 0.1M NaCl.

Dissociation constant K<sub>i</sub> (μM) of each compound was calculated from the following equation.

$$K_i = IC_{50} / (1 + ([C] / K_d))$$

IC<sub>50</sub> : The concentration of the compound of the present invention which inhibits half of the specific binding of [<sup>3</sup>H]PGE<sub>2</sub>

C : The concentration of [<sup>3</sup>H]PGE<sub>2</sub>

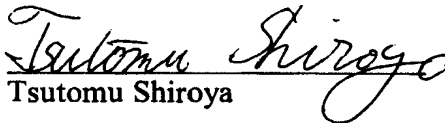
K<sub>d</sub> : The dissociation constant of [<sup>3</sup>H]PGE<sub>2</sub>

The results are shown below.

Example No.	K <sub>i</sub> (nM) EP <sub>2</sub>
6(32)	0.5
6(48)	3.5
6(53)	1.0
6(60)	0.4
6(63)	0.4
6(74)	1.5
6(77)	0.4
6(89)	0.6

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: August 30, 2007

  
Tsutomu Shiroya